

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA  
TRALKOXYDIM

Chemical Code # 5457, Tolerance # 52550

Original date 8/10/99

Revised date 11/10/99

I. DATA GAP STATUS

Chronic toxicity, rat: No data gap, possible adverse effect

Chronic toxicity, dog: No data gap, no adverse effect

Oncogenicity, rat: No data gap, possible adverse effect

Oncogenicity, hamster\*: No data gap, no adverse effect

Reproduction, rat: Data gap, no adverse effect indicated

Teratology, rat: No data gap, no adverse effect

Teratology, rabbit: No data gap, no adverse effect

Gene mutation: No data gap, no adverse effect

Chromosome effects: No data gap, no adverse effect

DNA damage: No data gap, no adverse effect

Neurotoxicity: Not required at this time

Metabolism No data gap, no adverse effect

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\*Syrian hamster was used for the second rodent species due to a characteristic liver porphyria found in tralkoxydim-treated mice (see Document No. 52550-010, Record No.165732 under "Oncogenicity, hamster", below), which would have limited the dose range in this species.

Toxicology one-liners are attached.

All record numbers for the above study types through 171338 (Document No.52550-036) were examined. This includes all relevant studies indexed by DPR as of 11/10/99.

In the 1-liners below:

\*\* indicates an acceptable study.

**Bold face** indicates a possible adverse effect.

File name: T991110.wpd

Original Summary by C. Aldous, August 10, 1999.

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

### COMBINED, RAT

**\*\*52550-011 165733** Stonard, M. D., "First revision to Tralkoxydim: 2-year feeding study in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 6/8/94. Report # CTL/P/1996. Fifty-two Alpk:APfSD rats/sex/group received 0, 50, 500, or 2500 ppm tralkoxydim (92.4%) in diet in a 2-year study. An additional 12/sex/group were treated for 1 year prior to necropsy. Essential elements of a "combined" study were evaluated. NOEL = 50 ppm (estimated 2.9 mg/kg/day), based on decreased plasma cholesterol in females. **"Possible adverse effects"** (especially bilateral retinal atrophy in females, and testicular interstitial cell hyperplasia and benign tumors in males). Other findings at 2500 ppm included decreased body weight and food consumption, modest but consistent reductions in RBC parameters, increased lymphocyte counts (both sexes, first year only), and epididymal changes of reduced spermatozoa and nucleated (immature) germ cells. No related findings were noted at 500 ppm or below. Significant changes in liver function were indicated by greatly increased incidences of hepatocellular clear cells in both sexes and by markedly reduced cholesterol levels at 2500 ppm. Alanine aminotransferase was consistently elevated in high dose females. Age-related kidney changes common in *ad lib.* fed rats, such as chronic progressive glomerulonephropathy, were reduced in incidence or degree at 2500 ppm. High dose male survival was appreciably better than that of controls or low dose males, largely due to reduction in deaths attributed to chronic progressive glomerulonephropathy. It cannot now be determined whether the retinal lesions and male reproductive toxicity represented primary or secondary effects of test article. Study was not originally accepted, but was upgraded in the November 1999 re-examination, considering supplemental data (records #171336 and #171337, below). The primary acceptability issue was the need for a reconciliation between ophthalmology and histopathology segments of the report. Aldous, 8/9/99, 11/9/99.

52550-036 171336, 171337 (Addendum to 52550-011 165733, Report # CTL/P/1996). The DPR review sought clarification as to why retinal atrophy was observed in histopathology, but not evident in ophthalmology. Response noted that retinal atrophy typically originates at the periphery of the retina, making it difficult to see during ophthalmologic examination. The lesion is usually focal in nature. Only when the lesion progresses to proliferation of glial cells, creating a white discoloration, is the lesion readily evident by ophthalmology. Generally histopathology is thus more sensitive than ophthalmology for lesions of this type. The response also addressed the Leydig cell tumors, which were identified as "possible adverse effects" in the DPR review. This addendum noted that meaningful increases in these tumors were limited to the highest dose group, and were late in onset. Leydig cell tumors are rare in humans, and there are important differences between rat and man in hormonal responses of Leydig cells. Investigators postulated that alterations in the hypothalamic/pituitary/gonadal feedback loop may have influenced tumor response. Cholesterol is a precursor to testosterone production, and levels of cholesterol were remarkably reduced at the high dose level. Thus investigators postulated that limitation of cholesterol substrate may have reduced circulating testosterone levels, prompting over-stimulation of Leydig cells via the feedback loop. Investigators noted that a review article by E. D. Clegg *et al.* (1997) discusses limitations of rat Leydig cell tumors as predictors of human oncogenic risks. The study is now **acceptable**. Data still indicate "possible adverse effects",

however this supplement provides useful perspectives on the interpretation of the Leydig cell tumor increase. Aldous, 11/9/99.

#### CHRONIC TOXICITY, RAT (See "Combined, Rat", above)

#### CHRONIC TOXICITY, DOG

**\*\*52550-012 165734** Stonard, M. D., "First revision to Tralkoxydim: 1-year oral dosing study in dogs", Zeneca Central Toxicology Laboratory, Alderley Park, 4/27/94. Laboratory Report # CTL/P/1794. Four beagles/sex/dose were administered tralkoxydim (94.9% purity) by gelatin capsule at 0, 0.5, 5.0, or 50 mg/kg/day in a 1-yr chronic study. NOEL = 0.5 mg/kg/day (fatty liver accompanied by high alanine aminotransferase activity in one male at 5.0 mg/kg/day). Also, alkaline phosphatase was generally elevated in 5.0 mg/kg/day females. Common findings at 50 mg/kg/day were elevated alkaline phosphatase and alanine aminotransferase, decreases in plasma cholesterol, triglycerides, and albumin, elevated organ weights (liver, adrenals, and thyroids), gross liver findings (swollen lobes, accentuated lobular pattern, mottled, friable), liver fatty change, adrenal gland vacuolation (zona fasciculata and zona reticularis). Gall bladder was discolored at 50 mg/kg/day, but there was no associated histopathology. RBC parameters were reduced slightly at 50 mg/kg/day, particularly red cell counts in males. No adverse effects. Acceptable with deficiencies as identified in the review. Aldous, 3/23/99.

#### ONCOGENICITY, RAT (See "Combined, Rat", above)

#### ONCOGENICITY, HAMSTER

**\*\*52550-013 165735** Stonard, M. D., "Tralkoxydim: Lifetime feeding study in the hamster", Zeneca Central Toxicology Laboratory, Alderley Park, 6/8/94. Report # CTL/P/2362. Syrian hamsters, strain LakLVG(SYR), were dosed in diet with 0, 250, 2500, or 7500 ppm tralkoxydim (purity 97.6%) for up to 79 weeks. There were 72/sex/group in treated groups, and 3 control groups/sex of the same size due to a comparatively small historical control database. Mortality was high in all female groups, largely due to an enteropathy characteristic of the species. The comparatively short life spans may have contributed to the low overall incidences of tumors. There was no treatment effect on oncogenicity. NOEL = 250 ppm (M: 14.9 mg/kg/day, F: 14.8 mg/kg/day: based on lipofuscin accumulation in hepatocytes of both sexes, and in Kupffer cells of females). Acceptable, with no adverse effects. Aldous, 5/18/99.

**52550-010 165732** Manley, A., "Tralkoxydim: An overview in support of the use of the hamster for oncogenicity testing." That overview notes that three strains of mice shared a common response to dose levels as low as 25 ppm tralkoxydim: . . . "areas of either focal or multifocal necrosis, hyperplasia of the bile ducts, peri biliary fibrosis, with an associated acute inflammatory reaction and yellow/brown pigment accumulation in the bile ducts, hepatocytes and Kupffer cells" (p. 8 of that record). The pigment was further evaluated and determined to be protoporphyrin IX. This symptomology seems to be unique to mice: no comparable findings were obtained with rats (Alpk:AP strain), Dunkin Hartley guinea pigs, Syrian hamsters, dogs, or marmoset monkeys. This record also briefly describes the 90-day study in Syrian hamsters, in

which the highest dose levels of 10000 to 20000 ppm elicited exacerbated the extent of nephropathies common to the species. Aldous, 2/11/99 (no worksheet).

## REPRODUCTION, RAT

52550-014 165737 Wickramaratne, G. A., "First revision to Tralkoxydim: Multigeneration study in the rat", Zeneca Central Toxicology Laboratory, 6/7/94, Laboratory Report #CTL/P/1932. Alpk:APfSD (Wistar-derived) rats, 15 males and 30 females per group, were dosed in diet with 0, 50, 200, or 1000 ppm of tralkoxydim. Treatment was continuous over 3 generations, with one littering period per generation. Mating was 1:2 M:F after pre-mating periods of 12 weeks (F0 generation) or at least 11 weeks (F1 and F2 parental generations). Study design was generally consistent with 1982 U.S. EPA guidelines, except that litters were not culled to standardize litter sizes, and (most importantly) the study lacked full histopathology on reproductive tissues (generally only grossly abnormal tissues or reproductive tracts of suspected infertile rats were examined). No adverse effects were indicated. Parental NOEL = 200 ppm (modest decrements in body weight and food consumption). Developmental NOEL = 200 [slightly reduced pup weights, and possible treatment-related increase in hydronephrosis (unilateral)]. Study is unacceptable, and possibly upgradeable (guideline-mandated reproductive tissues of all control and high dose F0 and F1 rats need to be examined for histopathology). Aldous, 8/9/99. (See also 1-liner for Record No. 171338, below).

52550-014 165736 Summary for the pilot study for the primary study, 52550-014 165737, above (cited in the primary study review).

52550-036 171338 (Addendum to 52550-014 165737, Study # CTL/P/1932). The DPR review stated that "guideline-mandated reproductive tissues of all control and high dose F0 and F1 rats need to be examined for histopathology". A brief response stated that (1) the study met OECD regulations at the time of the study commencement, (2) there was no systematic histopathological examination of control and high dose rat reproductive tissues, since there were no adverse reproductive effects identified, (3) tissues were examined for on-study deaths and for gross lesions, with no effects evident, (4) data from the chronic rat study should cover the need for histopathology of F0 rats, and (5) major regulatory agencies have accepted this study. The DPR response noted that the U.S. EPA guidelines, which call for histopathology of F0 and F1 parental rats, were published about 3 years before the commencement of this study, that far too few tissues were examined in this study to draw meaningful conclusions, and therefore that histopathology data from F1 parental rats would be needed to fill this data requirement. Aldous, 11/9/99.

## TERATOLOGY, RAT

\*\*52550-016 165740 Pigott, G. H., "PP604: Second teratogenicity study in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 9 June 1994, Report # CTL/P/1423. Worksheet contains essential data from a previous study of virtually identical design except for dosing levels: 52550-015 165739 Moxon, M. E., G. H. Pigott, P. B. Banham, and I. Pate, "First amendment to PP604: Teratogenicity study in the rat", Zeneca Central Toxicology Laboratory, Alderley Park, 13 June 1994, Report # CTL/P/1332. Both studies used groups of 24 Alpk:AP dams/group, dosed on days 7-16 [Gestation day 1 = day of positive vaginal smear], with

sacrifice on day 22. Dose levels were 0, 0.5, 1, 3, and 300 mg/kg/day in Record No. 165740 and 0, 3, 30, and 300 mg/kg/day in Record No. 165739. Test article was tralkoxydim, 96.4%, in volume of 10 ml corn oil/kg bw by gavage. Maternal NOEL = 30 mg/kg/day (4/24 maternal deaths in each of the two studies; piloerection, hunched and/or thin appearance, and signs of urinary incontinence at 200 mg/kg/day; a general increase in range and frequency of findings at 300 mg/kg/day). Developmental NOEL = 3 mg/kg/day (slight ossification delays at 30 mg/kg/day). A dose of 200 mg/kg/day elicited a reduction in mean fetal weight and a marginal increase in late intrauterine deaths. At 300 mg/kg/day, these findings were more pronounced, also five dams had total litter resorptions, and there was a statistically significant decrease in mean live litter size. Vertebral malformations, particularly fused and misshapen centra, were characteristic high dose events with marked dose-response at 200 to 300 mg/kg/day: litter (fetal) incidences were 8 (13) at 200 mg/kg/day and 14 (60) at 300 mg/kg/day ("possible adverse effects"). Soft tissue findings of subcutaneous edema (torso) and anasarca were significantly elevated at 300 mg/kg/day. The most definitive changes at 30 mg/kg/day were ossification delays in 1<sup>st</sup> and 2<sup>nd</sup> cervical vertebrae and slightly elevated manus/pes scores (i.e. poorer ossification in metacarpals, metatarsals, and phalanges). Acceptable. Aldous, 5/26/99.

52550-015 165738 A brief introduction to the rat teratology studies under Record Nos. 165739 and 165740, above. No worksheet is needed.

#### TERATOLOGY, RABBIT

\*\*52550-032 165827 Killick, M. E., G. A. Wickramaratne, P. B. Banham, and I. Pate, "First revision to PP604: teratology study in the rabbit", Zeneca Central Toxicology Laboratory, Alderley Park, 6/13/94. Laboratory Report # CTL/P/1373. [PP604 = tralkoxydim]. Eighteen NZW rabbits/group were dosed by gavage on days 7-19 at 0, 2.5, 20, or 100 mg/kg/day. The high dose level appeared justifiable, based on the pilot study in Record No. 165826 in this same volume, however the high dose proved to be excessively toxic to the dams (often these does appeared "subdued", had few or no feces, and/or appeared "thin" prior to the day of abortion). Unfortunately, investigators employed an unnecessarily large spread of dose levels, greatly reducing the utility of this study due to a lack of a dosage group just below the level of frank maternal toxicity. The study is acceptable, with the above limitation. Maternal NOEL = 20 mg/kg/day [8 abortions plus one moribund sacrifice at 100 mg/kg/day, with evidence of hemorrhage in stomach in most decedents]. Developmental NOEL = 20 mg/kg/day (increased late post-implantation losses). No adverse developmental effects in the absence of excessive maternal toxicity. Aldous, 5/19/99.

#### GENE MUTATION

\*\*52550-017 165741 Callander, R., "PP604: An evaluation in the Salmonella mutagenicity test", ICI Central Toxicology Laboratory, Macclesfield, 1 May 1986. Laboratory Report # CTL/P/1495. *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 were used in an Ames-style plate incorporation assay with and without S9. Tralkoxydim dose levels were uniformly 1.6, 8, 40, 200, 1000, and 5000 µg/plate, and each level was run in triplicate in each of two tests. Positive controls were functional. Tralkoxydim was negative in each situation. Acceptable, with no adverse effects. Aldous, 5/27/99.

\*\*52550-017 165742 Clay, P., "PP604: Assessment of mutagenic potential using L5178Y mouse lymphoma cells", ICI Central Toxicology Laboratory, Macclesfield, 11/4/85. Cell

suspension counts at 48 hr are given in Record No. 165743 in this volume. Laboratory Report # CTL/P/1401. Cell suspensions were exposed to concentrations of 25, 50, 100, 200, or 400 µg/ml of tralkoxydim, purity 97.8%, for 2 hr (with and without S9 activation) prior to a 48 hr expression time, followed by exposure to media selective for forward mutations. The first of two experiments yielded poor cell survival under all conditions, and is of questionable value. The second experiment provided plausible survival data. Results do not indicate a treatment effect. Acceptable with deficiencies as indicated. Aldous, 8/9/99.

### CHROMOSOME EFFECTS

\*\*52550-017 165744 Wildgoose, J., I. Braithwaite, C. Howard, and C. Richardson, "PP604: A cytogenetic study in human lymphocytes *in vitro*", ICI Central Toxicology Laboratory, Macclesfield, 10/30/85. Laboratory Report No. CTL/P/1395. Lymphocytes from 2 donors were used to evaluate effects on abnormal cell counts with and without S9 activation. Tralkoxydim levels of 25, 125, and 250 µg/ml were utilized, along with negative (DMSO) controls and appropriate positive controls. High background incidence of chromosomal aberrations made data from one of the donors unsuitable for analyses. Data from the remaining donor, representing counts of 200 cells/treatment level with and without S9, showed no signs of a treatment effect. Acceptable, with no adverse effects indicated. Aldous, 6/1/99.

### DNA DAMAGE

\*\*52550-017 165745 Sheldon, T., C. Richardson, J. Shaw, and G. Barber, "PP604: An evaluation in the mouse micronucleus test", ICI Central Toxicology Laboratory, Macclesfield, 4/29/86. Laboratory Report # CTL/P/1385. C57BL/6J/Alpk mice, 5/sex/treatment/time period, were dosed once ip with tralkoxydim at 300 or 480 mg/kg, negative control (corn oil vehicle), or positive control (cyclophosphamide), with sacrifice at 24, 48, or 72 hr. Tralkoxydim was negative at all but one treatment combination (males, 24 hr sacrifice, 480 mg/kg). A focused repeat study examined routine numbers of PCE's of males and females at 24 and 48 hr, and provided extensive counts (5000 PCE's each) on males only (controls and both tralkoxydim levels) at 24 hr only. No treatment effect on micronuclei was confirmed, and the overall study is considered negative. Acceptable, with no adverse effects. Aldous, 6/2/99.

\*\*52550-017 165746 Trueman, R., "Tralkoxydim: Assessment for the induction of unscheduled DNA synthesis in rat hepatocytes *in vivo*", ICI Central Toxicology Laboratory, Macclesfield, 9/23/87. Laboratory Report # CTL/P/1842. Male Alpk:AP rats, 5/dose/time interval, were dosed with 250, 500, or 1000 mg/kg tralkoxydim by gavage either 4 hr or 12 hr before sacrifice. Positive controls, 6-*p*-dimethylaminophenylazobenzthiazole ("6BT", 40 mg/kg), and negative controls (corn oil) utilized 2 rats/time period. Hepatocytes were collected after collagenase perfusion. Cells were allowed to attach to glass coverslips, exposed to labeled <sup>3</sup>H-thymidine, followed by removing the media and replacing labeled thymidine with unlabeled thymidine. Following fixation, automated nuclear grain counting was performed. Tralkoxydim did not affect counts, whereas controls were functional. Acceptable, with no adverse effects. Aldous, 6/2/99.

### NEUROTOXICITY (Not required at this time)

## METABOLISM

Summary: The data below show that tralkoxydim is efficiently absorbed and rapidly excreted in rats (the primary test species) and hamsters. Tissue levels were consistently low. In rats, the size of dose (1 or 40 mg/kg) had no apparent bearing on disposition of tralkoxydim. The dominant initial route of excretion is in the bile. In intact rats there is extensive enterohepatic recirculation, leading to 50% or more of residues being excreted in urine. The primary metabolite is tralkoxydim acid, formed by oxidation of the methyl group on the phenyl group *para* to the cyclohexene moiety. Often an additional methyl group on the tralkoxydim phenyl ring is oxidized to an alcohol. Some additional metabolism at the imino portion of the molecule was observed, forming respective oxazoles. Information is sufficient to characterize disposition of tralkoxydim. A human study found tralkoxydim acid to be the major metabolite. The latter study was intended only to evaluate whether tralkoxydim acid could be used as an indicator of tralkoxydim exposure, and was successful to that end. Aldous, 6/11/99.

52550-018 165747 Prout, M. S., and E. F. Howard, "First revision to PP604: Excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat", ICI Central Toxicology Laboratory, Macclesfield, 5/12/94. Laboratory Report # CTL/P/1393. Alpk:AP rats, 5/sex, were dosed once by gavage with 1 mg/kg tralkoxydim (<sup>14</sup>C-labeled in the phenyl ring) in corn oil. Urine and feces were collected daily. Blood, liver, kidney, and fat were collected at termination. Samples (except urine) were combusted and carbon was trapped for scintillation counting. Portions of urine and fecal samples were separated by TLC and chromatograms were compared to the retention time of tralkoxydim. Metabolites were not characterized in this report. Excretion was rapid and virtually quantitative. Ratios of radioactivity in urine and feces were about 2:1 in males and 1:1 in females. There was no detectable tralkoxydim found in chromatograms of urine samples, and only small percentages of radioactivity co-eluting with tralkoxydim in fecal sample extracts. Of tissues assayed, only kidney provided readings at or above the limits of detection (0.01 µg/g tissue). Investigators calculated that kidney residues were less than 0.1% of administered dose. This portion of the metabolism data is valid and acceptable. Aldous, 6/4/99.

52550-018 165748 Prout, M. S., and E. F. Howard, "First revision to tralkoxydim: excretion and tissue retention of a single oral dose (1 mg/kg) in the hamster", ICI Central Toxicology Laboratory, Macclesfield, 6/17/94. Laboratory Report # CTL/P/1627. Syrian hamsters, 5/sex, were dosed once by gavage with 1 mg/kg tralkoxydim (<sup>14</sup>C-labeled in the phenyl ring) in corn oil. Urine and feces were collected daily until termination at day 7. Major tissues were analyzed for residues. Samples (except urine) were combusted and carbon was trapped for scintillation counting. Portions of urine and fecal samples were analyzed by TLC. Metabolites were not characterized, however tentative identifications of the major peaks in urinary chromatograms were consistent with tralkoxydim acid, and lesser amounts of the tralkoxydim acid oxazole. Recoverable residues were about 4-fold higher in urine than in feces for either sex. Detectable organ concentrations were limited to liver, kidney, and blood. Investigators calculated that liver residues were about 0.04% of administered dose. Kidney and blood accounted for much lower percentages of dose. This portion of the metabolism data is valid and acceptable. Aldous, 6/4/99.

52550-018 165749 Prout, M. S., E. F. Howard, and A. Soames, "First revision to PP604: excretion and tissue distribution of a single oral dose (40 mg/kg) in the rat", ICI Central Toxicology Laboratory, Macclesfield, 5/16/94. Laboratory Report # CTL/P/1482. Alpk:AP rats were dosed with 40 mg/kg tralkoxydim (<sup>14</sup>C-labeled in the phenyl ring) in either a 7-day excretion balance study (5/sex) or an autoradiography study (1/sex at 5 hr and 1/sex at 48 hr after dosing, with radioassay of exhaled CO<sub>2</sub> for the first 24 hours in the latter group). The autoradiography study

found no label in exhaled CO<sub>2</sub>, and autoradiograms indicated rapid elimination of label from the body. Blood, liver, kidneys, and fat were collected at termination of the excretion balance study. Tissues and excretion product samples (except urine) were combusted, and carbon was trapped for scintillation counting. Ratios of radioactivity in urine and feces were about 2:1 in males and 1:1 in females. Assayed tissues had very low radioactivity: liver accounted for the greatest percentage of administered dose (only about 0.02%). This portion of the metabolism data is valid and acceptable. Aldous, 6/4/99.

52550-018 165750 Bratt, H., "First revision to tralkoxydim: repeat dose (1 mg/kg) study in the rat", Zeneca Central Toxicology Laboratory, Macclesfield, 4/25/94. Laboratory Report # CTL/P/2121. Alp:APfSD rats, 6/sex, were dosed by gavage daily for 14 days with 1 mg/kg/day (purity 97.8%) unlabeled tralkoxydim. On the 15<sup>th</sup> day, 4 rats/sex received 1 mg/kg/day tralkoxydim (<sup>14</sup>C-labeled on the cyclohexene ring). Urine and feces were collected at 24-hr intervals. Various tissues were assayed at termination (7 days). Samples (except urine) were combusted prior to carbon trapping for scintillation counting. Portions of urine and fecal samples were separated by TLC. Metabolites were provisionally identified on the basis of TLC mobility. As was the case with single radio-labeled doses at 1 or at 40 mg/kg tralkoxydim, excretion was rapid (about 86% of dose excreted within 24 hr in males vs. 48% in females), with males excreting somewhat higher percentage of dose in urine than females (61% vs. 52%). Only a trace of unaltered tralkoxydim was found in urine or feces. Provisional identification of metabolites indicated tralkoxydim acid as the major metabolite in both sexes (70-71% of recovered label in urine, and 43-56% of label in feces). Other major urinary metabolites were tralkoxydim alcohol (5% in M, 22% in F), tralkoxydim diol (13% in M, 1% in F), and tralkoxydim alcohol oxazole (7% in M, 3% in F). These metabolites were also the most common fecal residues: tralkoxydim alcohol (28% in M, 12% in F), tralkoxydim diol (4% in M, 7% in F), and tralkoxydim alcohol oxazole (11% in M, 12% in F). Tissue concentrations were uniformly low: the most notable being liver, representing about 0.02 to 0.03% of total dose. This portion of the metabolism data is valid and acceptable. Aldous, 6/7/99.

52550-018 165751 Prout, M. S., "First revision to Tralkoxydim: biotransformation in the rat", Zeneca Central Toxicology Laboratory, Macclesfield, 4/25/94, Laboratory Report # CTL/P/1907. Alp:AP rats, 2/sex, were fitted with biliary fistulas in order to determine the relative importance of various routes of excretion. Fistulated rats were dosed with <sup>14</sup>C-phenyl labeled tralkoxydim, and excretion profiles were evaluated over 2 days. Over 80% of radioactive residues recovered were found in the bile, with most of the remainder in urine. Over 80% of biliary residues were determined to be tralkoxydim acid. While tralkoxydim acid was also the largest single product of tralkoxydim metabolism in non-fistulated rats, rats without fistulas had much higher proportions of several other metabolites, including tralkoxydim alcohol, tralkoxydim diol (found only in males), and the oxazoles of tralkoxydim acid or alcohol. Results show that urinary metabolites arose principally from enterohepatic recirculation, and suggest that further metabolism occurred during this process. This record briefly describes techniques for separation and identification of metabolites, showing that metabolism involved oxidation of tralkoxydim aromatic ring substituents, yielding tralkoxydim acid, alcohol and diol products. The primary site of oxidation is the methyl group on the aromatic ring *para* to the cyclohexene moiety. Often one of the methyl groups *ortho* to the cyclohexene moiety was subsequently oxidized to the corresponding alcohol. Some additional metabolism at the imino portion of the molecule was observed, forming respective oxazoles. Data suffice to characterize the major metabolites of tralkoxydim in rats. This portion of the metabolism data is valid and acceptable. Aldous, 6/10/99.



52550-018 165752 Woollen, B. H., J. R. Marsh, and M. F. Wilks, "Tralkoxydim: pharmacokinetics in man following a single oral dose", Zeneca Central Toxicology Laboratory, Macclesfield, 6/20/91. Laboratory Report # CTL/R/1073. Seven male volunteers were dosed once with 5 mg tralkoxydim in corn oil, provided in gelatin capsules. Blood and urine were monitored daily for tralkoxydim acid. Quantitation in urine was by GLC using electron capture following alkaline hydrolysis, extraction into diethyl ether, conversion by heating in hot ethanol to form the oxazole derivative on the cyclohexenone ring, and derivatization of the acid moiety to the pentafluoropropionyl ester. Report did not indicate that any other metabolites were assayed. Tralkoxydim acid in urine constituted about 41 to 71% of administered dose. Assays of tralkoxydim acid in blood (sample preparation not described) reported no measurable residues. Investigators concluded that assay for tralkoxydim acid could serve as an index of absorption of tralkoxydim in man. Useful ancillary data, not applicable to data requirements. Aldous, 6/10/99.

#### SUBACUTE STUDIES TO EVALUATE MOUSE LIVER PATHOLOGY (includes studies in other species for comparison)

52550-007 165721 Stonard, M. D., "Tralkoxydim: 14 day species comparison feeding study", ICI Central Toxicology Laboratory, Alderley Park, 10/10/89. Laboratory Study # CTL/P/2633. Five males/species/dose were dosed in diet for 14 days with 0, 50, or 500 ppm tralkoxydim. Test species were Alpk:APfSD rats, C57BL/10JfCD-1/Alpk mice, and LakLVG(SYR) hamsters. Also, Alpk:Dunkin Hartley guinea pigs were dosed at 0, 100, and 1000 ppm. Estimated dose levels for 50 and 500 ppm animals were 6.4 and 61 mg/kg/day (rats), 9.7 and 108 mg/kg/day (mice), 6.0 and 57 mg/kg/day (hamsters), and 5.5 and 54.2 mg/kg/day (guinea pigs). Mice had strong, dose-related increases in ALP, ALT, and cholesterol. Guinea pigs had elevated cholesterol at 1000 ppm only. Mice had markedly elevated total liver porphyrins at both dose levels. Mouse liver weights were elevated at both dose levels. All treated mice had dark, discolored livers. Mouse liver histopathology in both groups (commonly dose-related in incidence or intensity) included bile duct hyperplasia, biliary fibrosis, portal inflammation, pigment accumulations in hepatocytes, Kupffer cells, and bile ducts, pigment birefringence under polarized light, and pigment fluorescence under uv light. High dose mice had increased hepatocyte mitotic rates. Data indicate that mice are uniquely sensitive to porphyrin-associated liver pathology. Aldous, 8/9/99.

52550-007 165722 Stonard, M. D., "Tralkoxydim: 14-day oral gavage study in the marmoset", ICI Central Toxicology Laboratory, Alderley Park, 10/12/89. Laboratory Study # CTL/P/2798. Three marmosets/sex/dose received 0, 10, or 100 mg/kg/day by gavage in 2.5 mg corn oil per kg bw. Clinical observations, hematology, clinical chemistry, and general necropsies were performed on all animals. Livers and adrenals were examined for histopathology. There were no clear indications of toxicity, although occasional instances of vomiting in one/sex at the high dose were considered by investigators to be treatment-related. There were no macroscopic or microscopic treatment effects. Aldous, 6/11/99.

52550-007 165723 Brady, A. M., and E. A. Lock, "Tralkoxydim: First revision to the mechanism of tralkoxydim-induced hepatic cholestasis: studies in rats and mice", Zeneca Central Toxicology Laboratory, Alderley Park, 6/14/99. Laboratory Study # CTL/R/1025. Investigators evaluated three primary parameters in preparations from mouse and rat livers at various times after dosing by gavage at various dose levels: total porphyrin in liver homogenates, 5-aminolevulinic acid synthetase (ALAS) in liver homogenates, and ferrochelatase in liver mitochondrial fractions. Measurable accumulation of porphyrin was seen at 2 mg/kg in mice after 24 hr, but was not seen in rats at dose levels up to 750 mg/kg. Higher doses reduced the time required before porphyrin

accumulation was evident in mice: 10 mg/kg tralkoxydim elevated porphyrin within 2 hr, and 100 mg/kg elevated porphyrin within 1 hr. Repeated dosing of 100 mg/kg/day in rats did not increase porphyrin levels, whereas porphyrin levels in mice appeared to increase systematically with time (sample sizes were small and SD's were large enough in mice so that this was not a sure treatment effect). There was an apparent dose-response for increased ALAS in mouse liver up to 100 mg/kg. Higher doses did not increase the response further. Rats appeared to have a slight increase in ALAS at 400 to 750 mg/kg. Ferrochelatase activity was not altered in rats up to 750 mg/kg, whereas mice achieved maximal inhibition at low dose levels (evidently about 10 to 20 mg/kg, based on the figure) through the high dose of 750 mg/kg. It took about 3 hr in mice to achieve maximal ferrochelatase activity inhibition. Data are consistent with the conclusion that ferrochelatase inhibition was the primary alteration caused by tralkoxydim, leading to reduced heme production and then compensatory increases in ALAS activity. Useful ancillary data. Aldous, 6/15/99.

52550-007 165724 Brady, A. M. and E. A. Lock, "Tralkoxydim: First revision to identification of an inhibitor of ferrochelatase in the livers of mice dosed with tralkoxydim", Zeneca Central Toxicology Laboratory, Alderley Park, 6/14/94. Laboratory Study # CTL/R/1038. Mice were dosed ip with <sup>14</sup>C-5-aminolevulinic acid (ALA) followed gavage dosing with arachis oil (control), tralkoxydim (50 or 200 mg/kg), or DDC (a known inhibitor of heme synthesis from ALA). Liver homogenates were applied to Sephadex columns, from which radio-labeled eluates were collected. Major peaks were (1) heme (all mice) and (2) a second peak, which was found only in tralkoxydim-treated mice and in mice dosed with "DDC", a compound known to block ferrochelatase activity by producing N-alkylated porphyrins which block heme production by competition with protoporphyrin IX (PPIX). Ferrochelatase activity was measured in liver mitochondrial fractions, and inhibition by the above column fractions was assayed. When the above radiolabel studies had determined which fractions contained a component which was a potent ferrochelatase activity inhibitor, this component was collected and purified from preparations collected from mice dosed with DDC, tralkoxydim (varied dose levels), or vehicle controls. The UV absorption spectrum of the ferrochelatase inhibitor was much like that of PPIX, but major peaks were offset by a few nm. UV absorbance maxima of this inhibitory porphyrin were virtually identical to those of authentic N-methyl PPIX. Highly purified inhibitory porphyrin from tralkoxydim-dosed mice generated an NMR peak consistent with N-methyl protons of N-methyl PPIX (unfortunately the report did not show a spectrum from normal PPIX). The tralkoxydim-associated porphyrin was methylated on the two carboxylic acid groups, then subjected to MS analysis. A major peak was consistent with N-methyl PPIX dimethyl ester (unfortunately the report did not show a spectrum from normal PPIX, similarly esterified). The amount of N-methyl PPIX produced after 25 mg/kg tralkoxydim was several-fold higher than in controls, and was not further increased at tralkoxydim dose levels up to 500 mg/kg. Thus this study gives strong evidence that N-methylation of PPIX leads to inhibition of ferrochelatase, resulting in hepatic porphyria in mice. Aldous, 8/9/99.

52550-007 165725 Brady, A. M. and E. A. Lock, "Tralkoxydim: First revision to the origin of N-methyl protoporphyrin IX in the liver of mice following administration of tralkoxydim", Zeneca Central Toxicology Laboratory, Alderley Park, 6/14/94. Laboratory Study # CTL/R/1040. Investigators utilized several <sup>14</sup>C-labeled analogs of tralkoxydim to determine the source of the methyl group which becomes transferred to protoporphyrin IX to create the N-methyl derivative which is porphyrinogenic in mice. Initial studies involved labeling either (1) the methyl group on the phenyl ring located *para* to the hexene ring, (2) the ethoxymine group, or (3) the C-ethyl group. Only in the case of labeled C-ethyl group was most of the N-methyl content of N-methyl protoporphyrin IX (N-CH<sub>3</sub> PPIX) derived from tralkoxydim. Further studies examined the impact of structural analogs at each of the 3 loci above. Any change in the alkyl group of the C-ethyl series

eliminated porphyrinogenic activity. Creation of an oxazole or isoxazole by closing the imine nitrogen into a ring also eliminated porphyrinogenic activity. Changes in the ethoxyimine series led to a range of measurable porphyrinogenic activities, with the O-methyl derivative being weakest and O-propyl being nearly comparable to tralkoxydim in porphyrinogenicity. Ferrochelatase inhibition patterns followed the patterns above for porphyrinogenicity: C-alkyl alterations did not inhibit ferrochelatase, whereas any of the alkoxyimines tested caused similar reductions of ferrochelatase activity, including the O-methyl derivative. Investigators were able to conclude that formation of N-CH<sub>3</sub> PPIX arises by direct alkylation rather than by stimulation of an endogenous pathway. Aldous, 6/16/99.

52550-007 165726 Brady, A. M. and E. A. Lock, "Tralkoxydim: first revision to species differences in tralkoxydim-induced hepatic porphyria: studies in rats", Zeneca Central Toxicology Laboratory, Alderley Park, 6/14/94. Laboratory Study # CTL/R/1043. Alpk:APfCD-1 rats were dosed by gavage with tralkoxydim at levels up to 100 mg/kg/day for 4 days or up to 200 mg/kg for single dose to study tralkoxydim-induced hepatic porphyria. Liver porphyrin content and liver mitochondrial ferrochelatase activity were unchanged by treatment. Rats were dosed ip with <sup>14</sup>C-5-aminolevulinic acid (ALA) followed by ip dosing with arachis oil (control), tralkoxydim, or DDC (a known inhibitor of heme synthesis from ALA). Liver homogenates were applied to Sephadex columns, from which radio-labeled eluates were collected. Major peaks were (1) heme (all rats) and (2) a second peak, which was found only in rats dosed with "DDC". This compound is known to block ferrochelatase activity by producing N-alkylated porphyrins, which block heme production by competition with protoporphyrin IX (PPIX). In contrast to these rat study results, a comparable procedure in mice (Record # 165724) showed that tralkoxydim also caused N-alkylation of heme components in mice. The Sephadex column fraction associated with the second peak of the rat liver homogenate DDC eluates had marked inhibitory influence on mouse or rat liver mitochondrial ferrochelatases: corresponding fractions derived from control and tralkoxydim-treated rat liver extract eluates did not elicit such inhibition. Thus there is a great species difference between rat and mouse, evidently based on the ability of the mouse to cause N-alkylation of PPIX and resulting hepatic porphyria. Aldous, 6/17/99.

52550-007 165727 Brady, A. M. and E. A. Lock, "Tralkoxydim: first revision to species differences in tralkoxydim-induced hepatic porphyria: studies in hamsters", Zeneca Central Toxicology Laboratory, Alderley Park, 6/14/94. Laboratory Study # CTL/R/1044. This study is similar in design and outcome to the comparable one in rats (Record No. 165726), except as follows. Test animals were male hamsters (Lak LVG (SYR)). There was a small but statistically significant increase in total liver porphyrin at a single dose of 750 mg/kg, but not after 4 repeated doses of 100 mg/kg/day. Hepatic 5-aminolevulinic acid synthetase (ALAS) activity appeared elevated over controls at all dose levels over 25 mg/kg. The apparent elevation in ALAS activity was small compared to that reported for mice and was similar to the marginal increase indicated for rats (see Fig. 4 of Record No. 165723). Hepatic ferrochelatase activity was not altered in liver mitochondrial preparations. Figure 4 appears to show a tiny peak of <sup>14</sup>C radioactivity in the eluate of the tralkoxydim 200 mg/kg group corresponding to the fraction in which the DDC group displayed a much larger peak [presumably N-alkylated PPIX, based on data from a mouse study (Record No. 165724)]. Further, small peaks indicating inhibition of mouse liver mitochondrial ferrochelatase activity were seen in eluates of 50 and 200 mg/kg tralkoxydim groups corresponding to the fraction in which the DDC group displayed a much larger peak. The tralkoxydim peak heights were not dose-related. Investigators did not consider there to be "significant" inhibition of ferrochelatase in the tralkoxydim eluates. If any of the above findings are treatment-related, they are very small compared to responses in mice, and clearly support major inter-species differences. Aldous, 6/17/99.

52550-007 165728 Pauli, B., S. Kennedy, and L. Bastien, "Tralkoxydim: Investigation into the potential hepatotoxic effects of tralkoxydim (ACHIEVE®) herbicide in Canadian small mammals", Environment Canada, Canadian Wildlife Service, Hull, Quebec, 1/19/96. Laboratory Project ID: ZA0710962. Wild test species were the deer mouse (*Peromyscus maniculatis*), white-footed mouse (*Peromyscus leucopus*), and meadow vole (*Microtus pennsylvanicus*). Swiss mice (known to produce liver porphyria in response to tralkoxydim), were tested for comparison. Protoporphyrin levels were measured in extracts from liver homogenates. Mice had great increases in protoporphyrin levels in response to positive control substance (DDC) and to 100 mg/kg tralkoxydim. None of the wild species showed plausibly treatment-related responses to tralkoxydim at dose levels up to 100 mg/kg. Of the test species, only the white-footed mouse showed increases in protoporphyrin levels in response to DDC, and these responses were small compared to those of laboratory mice. The demonstration of predicted responses in laboratory mice and lack of response in any of the wild rodents suggests that tralkoxydim is unlikely to be a threat to non-target animals expected to be exposed. No worksheet, since the study was not directed at human health issues and did not indicate adverse effects. Aldous, 6/18/99.